

Amendments to the Specification:

Amend the paragraph beginning at page 16, line 3, of the English language specification as follows.

After  $10^7$  Ba/F3 cells were washed twice with PBS and once with “OPTI-MEM1” (Gibco-BRL), the cells were suspended into 0.2 ml of “OPTI-MEM1.” Ten mg each of “pCMX-GCRER-IRES-CD24,” “pCMX-GCR  $\Delta$  (5-195)/ER-IRES-CD24,” and “pMX-GCR  $\Delta$  (5-195, 725-756)/ER-IRES-CD24” was added to the cells, and transformation was performed using ~~“Gene Pulser”~~ a GENE PULSER apparatus (BioRad) at 290V, 960 mF. After the transformation, the cells were cultured for two days in the RPMI medium containing 10% FCS and 10 U/ml mIL-3 (R&D SYSTEMS). After  $10^6$  cells were washed with 5% FCS/PBS, the cells were reacted with 1 mg/ml of the anti-CD24 antibody (Pharmingen) for 30 minutes at room temperature. The cells were then washed twice with 5% FCS/PBS, reacted with a 1:20 dilution of the PE-labeled anti-mouse antibody (DAKO) for 30 minutes at room temperature, and washed again twice with 5% FCS/PBS. The cells were suspended in 1 ml of 5 mg/ml propidium iodide/PBS, and the CD24 expression was analyzed by flow cytometry (Becton Dickinson) using a 585 nm detector. The CD24 expression was detected from a number of the cells into which “pCMX-GCR  $\Delta$  (5-195)/ER-IRES-CD24” had been introduced. In this experiment, the cells into which “pCMX-GCR  $\Delta$  (5-195)/ER” was introduced were used as a control against the cells having “pCMX-GCR  $\Delta$  (5-195)/ER-IRES-CD24” introduced. The

results are shown in Fig. 9 and Table 1. Note that the data contain the signal from propidium iodide that was used to detect the dead cells.

Amend the paragraph beginning at page 21, line 23, of the English language specification as follows.

All retroviral transduction experiments were performed in P2 facilities, according to the institutional recombinant DNA biosafety guidelines. BOSC23 cells were transfected with MSCV/GCRTmR-IRES-CD8a or MSCV/  $\Delta$  GCRTmR-IRES-CD8a using Lipofectamine (Life Technologies) and the viral supernatants were harvested on day two post-lipofection. Fibronectin-assisted transduction of Ba/F3 cells was carried out on 6-well plates precoated with ~~RetroNeectin~~ RETRONECTIN recombinant human fibronectin (Takara Shuzo, Otsu, Japan) according to standard procedures [Hanenberg H, et al., Nat. Med., (1996) 2:876-882]. After retroviral infection, the transduced Ba/F3 cells were selected with a Magnetic Cell Sorting (MACS) system (Miltenyi Biotech, Bergisch Gladbach, Germany). Aliquots of  $1 \times 10^7$  Ba/F3 cells were incubated with anti-CD8a antibody-conjugated microbeads, and CD8a-positive cells were recovered according to the manufacturer's protocol. The selected cells were analyzed for CD8a expression by fluorescence-activated cell sorting (FACS) with a fluorescein isothiocyanate (FITC)-labeled anti-murine CD8a antibody (Pharmingen, San Diego, CA). Successfully transduced Ba/F3 cells (BaF/GCRTmR and BaF/  $\Delta$  GCRTmR) were clones by limiting

dilution, with an initial incubation with IL-3 for six days followed by expansion with  $10^{-7}$  M Tm (Sigma, St. Louis, MO).

Amend the paragraph beginning at page 28, line 26, of the English language specification, as follows.

(1) Plasmid construction

All enzymes used were purchased from New England Biolabs Inc (Beverly, MA). A mammalian expression vector pCMX-MfasER (kindly provided by Dr A. Kakizuka, Kyoto University, Kyoto, Japan), which contains the sequence encoding the HBD of rat estrogen receptor (ER) was digested with BamHI and EcoRI. The BamHI-EcoRI fragment containing the ER-HBD was separated by agarose gel electrophoresis and electroelution. This fragment was subcloned into BamHI-EcoRI site of the plasmid ~~pBluescript~~ pBLUESCRIPT (TOYOBO, Japan) by ligation. Further, tamoxifen receptor (TmR) cDNA was derived from a retroviral vector MSCV-ΔGCRTmR-IRES-EGFP (kindly provided by Dr R. Xu, Jichi Medical School, Tochigi, Japan) which contains the sequence encoding the HBD of mouse TmR, by polymerase chain reaction (PCR) with primer A (5'-CTGGATCCGGGCACTTCAGGAGAC-3'; SEQ ID NO:3, creating a BamHI site) and primer B (5'-CTGTCGACCACTAGTAGGAGCTCTCA-3'; SEQ ID NO:4, creating a SalI site). This cDNA was subcloned into BamHI-SalI site of the ~~pBluescript~~ pBLUESCRIPT plasmid by ligation. On the other hand, a mammalian

expression vector pcDNA3.1-c-mpl (kindly provided by Dr M. Takatoku, Jichi Medical School, Tochigi, Japan) which contains the cDNA for human c-mpl between the EcoRI and XbaI sites was digested with EcoRI and SacI. The EcoRI-SacI fragment containing most of the extracellular domain of c-mpl was separated by agarose gel electrophoresis and electroelution. Further, the rest c-mpl cDNA between SacI site and the c-terminal cytoplasmic domain was constructed by PCR using the pcDNA-c-mpl as a template with primer C (5'-CCCACCTACCAAGGTCCCTGG-3'; SEQ ID NO:5) and primer D (5'-CGGGATCCAGAGGCTGCTGCCAATAG-3'; SEQ ID NO:6, creating a BamHI site). Then the murine phosphoglycerate kinase (pgk) promoter-neomycin phosphotransferase gene (neo) cassette (EcoRI-BamHI) in MSCV2.2 retrovirus (a gift from Dr. R.G. Hawley, University of Toronto, Canada) was replaced with the EcoRI-SacI fragment of c-mpl and the SacI-BamHI fragment of c-mpl by trimolecular ligation to construct MSCV-mpl. The ~~pBluescript-ER~~ pBLUESCRIPT-ER plasmid and the ~~pBluescript-TmR~~ pBLUESCRIPT-TmR plasmid were digested with BamHI and SalI, and the ER and TmR fragments were separated by agarose gel electrophoresis and electroelution. These fragments were cloned into BamHI-SalI site of MSCV-mpl by ligation. The resultant vectors were designated as MSCV-mpl-ER or MSCV-mpl-TmR.

Amend the paragraph beginning at page 34, line 4, of the English language specification, as follows.

(4) In vitro clonogenic progenitor assay

The transduced and untransduced BM cells were harvested, and  $1 \times 10^5$  cells each were plated in 35-mm dish with 1 ml ~~StemPro~~ STEMPRO medium (GIBCO-BRL) in the presence or absence of 100 ng/ml of rhTPO or  $10^{-7}$  M of E2. In some experiments, 100 ng/ml of rmSCF, 100 U/ml of rhIL-6, 100 ng/ml of rhTPO, and 2 U/ml of recombinant human erythropoietin (rhEpo; Chugai Pharmaceutical) were added to the cultures. After 10 days of incubation at 37°C in a humidified atmosphere of 5% CO<sub>2</sub> in air, colonies were scored using an inverted microscope.